Changes in Water Status of Peach Flower Buds During Endodormancy and Ecodormancy Measured by Differential Scanning Calorimetry and Nuclear Magnetic Resonance Spectroscopy

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Abstract. Physiological changes in 'Hakuho' peach [*Prunus persica* (L.) Hatsch] flower buds during endodormancy and ecodormancy were investigated based on their water status measured by differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) spectroscopy. The developmental stage in ecodormancy, which was estimated as the number of days between the sampling date for shoots and the bloom date after forcing, was dominated by absorption of water and was closely related to the water content per dry weight. Two types of water differing in spin-lattice relaxation time (T1) of protons were detected in the flower buds. Water with the shorter T1 was considered to be freezing water as well as that with a longer T1. Nonfreezing water can not be detected by NMR. The change in the longer T1 coincided with the change in the water percentage relative to bud fresh weight and reflected the physiological development in ecodormancy. The shorter T1 value started to increase shortly before rest break and may have some relation with the physiological change at rest break.

The buds of most deciduous trees show no visible growth soon after their formation in the autumn, even if the temperature is favorable for bloom (endodormancy). Endodormancy is broken by a certain amount of chilling temperature (Chandler and Tufts, 1934). The period following endodormancy and before bud break, when buds remain dormant because ambient temperatures are unfavorable for growth, is endodormancy.

Information on the developmental stages of buds is useful in horticulture for estimating the flowering date in orchards and determining the time when heating should be started for greenhouse forcing. The progress in the developmental stage cannot be determined clearly from the appearance of buds. However, some cellular components change throughout endo- and ecodormancy. For example, the abscisic acid (ABA) level, which has been studied in buds of several species, is highest at the time of leaf fall during endodormancy and decreases throughout ecodormancy (Corgun and Peyton, 1970; Harrison and Saunders, 1975; Mielke and Dennis, 1975; Wright, 1975; Seeley and Powell, 1981). It has also been reported that sour cherry flower buds accumulate starch during endodormancy (Felker et al., 1983), and desaturated membrane lipids in apple buds increase during endo- and ecodormancy (Wang and Faust, 1990). Since water is the main solvent for these cellular components responsible for physiological development, changes in the mobility of the water in bud tissues are expected to

accompany the physiological changes. We therefore investigated the water status of flower buds to detect the physiological changes occurring during development toward bud break.

The water status of biological samples can be measured by means of nondestructive methods, such as nuclear magnetic resonance (NMR) spectroscopy. The NMR relaxation times of water protons reflect the motion of water molecules in tissues (Mathur-De Vre, 1984). Several types of water with different relaxation times indicating differing molecular mobility are usually found in living samples. The water in different physical status is detected by differential scanning calorimetry (DSC) based on the melting or crystallization heat curve characteristics. Thus, we can identify water in living samples according to this property.

In this research we investigated the water status of peach flower buds by NMR and DSC during endodormancy and ecodormancy.

Materials and Methods

Plant materials Ten first-year shoots, 30 to 40 cm in length, were randomly collected twice a month from 5 Dec. 1989 to 26 Mar. 1990 from 9-year-old 'Hakuho' peach trees planted at the Fruit Tree Research Station in Tsukuba, Japan. Bloom date was 30 Mar. The temperature in the orchard was recorded with a platinum resistance thermometer placed ≈ 1.5 m above the ground.

Proton relaxation time measurement. The collected shoots were divided into three sets at each sampling time to obtain replicates for relaxation time measurement. Flower buds were taken from the central part of each shoot and placed in a 10-mm-diameter NMR sample-tube. Twenty five buds were used per replicate until early February. Because of the increase in bud

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volume, only 20 were used in late February, 15 in early March, and 3 in late March. The spin-lattice relaxation time (T1) of water protons in the flower buds was measured with a NMR spectrometer (FX-90Q; JEOL, Tokyo) by the inversion recovery (IR) method. With a 4500-Hz spectral width and a 3- to 5-sec pulse repetition time according to the T1, free induction decay (FID) was accumulated four times at 27°C, and 20 data points with different pulse intervals were collected for each sample for T1 calculation. The measurement time for a sample was ≈ 10 min. The number of water components with a different T1, the population of each water component, and each T1 were determined from the resultant relaxation curve by a nonlinear least squares method using a subroutine of simplex written by Kobayashi (1981) on a computer (Yoshida and Nose, 1987).

Measurement of dry weight and freezable and nonfreezable water contents. Flower buds used for the determination of T1 were weighed and dried at 80°C for 2 weeks. Their water content was calculated as the difference between fresh and dry weight.

The amount of freezable water in buds was measured once or twice monthly by DSC analysis according to the method of Hatakeyama et al. (1988). A bud was placed in a DSC cell, which was then sealed and weighed. The cell was then placed in a DSC 100 calorimeter (Seiko, Tokyo) linked with a SSC5000TA station. The instrument was calibrated using the heat of pure ice melting, 333 J/g. The sample was frozen at -150°C at a cooling rate of $5^\circ\text{C}/\text{min}$ and then the temperature was raised at the rate of $5^\circ\text{C}/\text{min}$ until it reached 60°C . The peak area of the thermograms was measured by assuming a linear base line between temperatures prior to and after melting. The amount of freezable water was calculated from the area by using a calibration constant. The bud was then pre-dried at 100°C and dried under vacuum at 115°C for 2 h. The decrease in weight after drying was taken as the loss of total water. The unfrozen water content was obtained as the difference between the total water content and the freezable water content.

Estimation of the developmental stage. The shoots, from which some of the flower buds were taken for the T1 and DSC measurements, were put in a pail of water in a dark room at $20 \pm 0.5^\circ\text{C}$ and the humidity maintained at $80\% \pm 10\%$. The number of shoots in bloom was determined every day. A shoot was considered to have bloomed when at least one of its buds had flowered. The bloom date was defined as the date on which 30% of the shoots had bloomed. The percentage of bloom was calculated as (the number of shoots in bloom after 50 days' forcing/the number of all forced shoots) $\times 100$.

Results

Temperature and developmental stage of buds. The moving average of the previous 5 days temperature was low until early February. The temperature increased rapidly in February and decreased again in early March. After this cold period the temperature increased gradually until April (Fig. 1).

The percentage of bloom after forcing increased rapidly and reached 100% in early February (Fig. 1), indicating that the flower buds completed rest and entered into ecodormancy between 24 Jan. and 5 Feb. The number of days between sampling and bloom date decreased, indicating progressive development of the buds during ecodormancy. The number of days required for bloom decreased in February and March, but the rate decreased in early March, at which time the orchard temperature was lower than in late February and mid-March (Fig. 1).

Fresh weight, dry weight and water content. The fresh weight of the flower buds was almost constant from December to early

Fig. 1. Changes in moving average of the previous five days temperature (----), the percentage of bloom after forcing (■) and the number of days between sampling and bloom date (□). Sample shoots were forced at 20°C . The percentage of bloom was calculated 50 days after forcing and bloom date was defined as the date on which 30% of the shoots had bloomed.

Fig. 2. Changes in fresh weight (■) and dry weight (□) of a peach flower bud

Fig. 3. Changes in water content (■) and water percentage as to fresh weight of a peach flower bud (□).

Fig. 4. DSC scan of a peach flower bud sampled in early December.

February, when their external appearance did not visibly change. The fresh weight increased slowly during February and rapidly in March (Fig. 2), which was accompanied by an increase in bud volume. The dry weight of the buds did not change greatly until early March, but increased after that (Fig. 2). Water content of the buds increased in February and the increase accelerated in late March (Fig. 3). This change was similar to that in the fresh weight. Water percentage expressed on a fresh weight basis increased slightly during endodormancy and increased rapidly in late February (Fig. 3). This dramatic change in water percentage occurred after endodormancy in early February and before the large increase in dry weight in late March. The rate of increase in the water percentage decreased in early March.

Freezable and nonfreezable water contents. One representative DSC scan is given in Fig 4. The onset temperature of the melting heat peaks was between -16.5 and -14.5°C and shifted in late February (Table 1).

The content of nonfreezable water was always less than that of frozen water. The percentage of unfrozen water relative to the total water decreased progressively from December to early March (Table 1). On the other hand, the amount of nonfreezable water per dry weight decreased until early January. The increase from late February (Table 1), was similar to the changes in the developmental stage and the water percentage during ecodormancy.

Water proton relaxation time. Two types of water were detected in the flower buds on NMR relaxation time analysis (Fig. 5). The water component with the longer T1 (T1L) was the major while the shorter T1 (T1S) was the minor component (Fig. 6). T1L increased slightly during endodormancy and increased greatly after early

February (Fig. 5). The change in T1L during ecodormancy coincided with progress in the developmental stage and an increase in the water percentage in the buds. T1S increased almost steadily from January, before endodormancy break, to March (Fig. 5). The increases in T1L and T1S were both lower in early March.

The content of the water with T1L (WL) was always larger than that of the water with T1S (WS), but the percentage of WL decreased as the time elapsed after early January, (Fig. 6). The difference between the percentage of WL and that of WS decreased sharply in late March just before bloom.

Discussion

The fresh weight of the buds, which did not change noticeably during endodormancy, began to increase in late February during ecodormancy and increased remarkably in late March. In contrast, the dry weight remained unchanged until early March. This indicates that ecodormancy can be divided into two phases. In the first phase, the buds begin to absorb water from vessels in the shoots. In the second phase, the dry weight increases rapidly in addition to the water absorption acceleration. Ashworth (1984) reported that xylem vessel elements are not observed within the floral primordia of peach buds dormant. Water enters into buds when vascular differentiation resumes and xylem continuity is established, relatively close to the time of bloom. This evidence suggests that vascular elements develop relatively earlier than other elements of the peach flower bud in ecodormancy.

Since the increase in water content began in February, earlier than the increase in the dry weight, the fresh weight percentage of water rose sharply in late February. This trend slowed in early March, when temperature was lower, but rapidly increased again in late March. This change reflected progress in the developmental stage of buds during ecodormancy as estimated from the bloom of the forced shoots. This suggests that progress in the developmental stage during ecodormancy is correlated with the increase in water content per dry matter, rather than with the increases in the dry weight and total water content in the buds.

The percentage of nonfreezable water relative to total water decreased while the freezing water content increased during endodormancy, during which time the total water content remained unchanged. These results support the suggestion that bound or structured water in apple buds changed into free water when the buds were chilled during endodormancy (Faust et al., 1991). It was demonstrated that intracellular water in tissues of very hardy species forms glass, which does not freeze (Hirsh et al., 1985), while nonfreezable water content per dry weight increased in midwinter (Vertucci and Stushnoff, 1992). But, in the present study, the unfrozen water content per dry weight increased from late February when the air temperature increased. It is possible that

Table 1. The onset temperature of melting heat peaks, the percentage of nonfreezing water relative to total water and the amount of nonfreezable water per dry weight for peach buds sampled at different times during endodormancy and ecodormancy. The nonfreezable water content was obtained as the difference between the total water content and the frozen water content determined by DSC analysis.

Date	Onset temperature of melting (°C)	Nonfreezable water		Nonfreezable water	
		(% of total water)	SE	(dry weight)	SE
Early Dec.	-16.5	23.6	0.04	0.32	0.009
Early Jan.	-16.5	18.2	0.11	0.24	0.037
Early Feb.	-16.5	17.1	0.36	0.24	0.007
Late Feb.	-15.0	17.9	0.56	0.35	0.011
Early Mar.	-15.0	14.3	0.26	0.35	0.007
Late Mar.	-14.5	14.7	2.27	0.56	0.089

Fig. 5. Changes in spin-lattice relaxation time (T1) of water protons in peach flower buds during endodormancy and ecodormancy. A water component with a longer T1 (T1L, ■) and a shorter T1 (T1S, □) were detected.

Fig. 6. Changes in the percentage of the water component with a longer T1 (WL, ■) and that with a shorter T1 (WS, □) in peach flower buds during endodormancy and ecodormancy.

the unfrozen water that increased after early February may not be glass that is formed to survive low temperature, but another type of water.

Different methods of calculating the amount of unfrozen water have been proposed in DSC experiments. In some DSC studies, calculations have been based on assuming that the heat of melting of water in solutions is 333 J·g⁻¹ which is the heat of melting of pure water (Samygin and Livshin, 1970; Hatakeyama et al., 1988). Recent studies have proposed another method of calculation of the amount of nonfreezable water (Vertucci and Stushnoff, 1992) based on the demonstration that the heat of melting of water depends on the melting temperature (Gekko and Satake, 1981) and the water content of the sample (Vertucci, 1990). In the present study, however, we calculated the amount of nonfreezable water using the heat of melting of water in buds as that of pure water. All samples had water contents above 53%.

Two types of water (WL and WS) with different T1 (T1L and T1S) were measured in the buds throughout endo- and ecodormancy. T1L was 260 to 600 ms and T1S was 30 to 180 ms. The percentage of WS in the buds increased after early January, the increase being especially large just before bloom. Some reports of plant tissue water measured by NMR concluded that short relaxation times are related to water bound to the cell wall and vessels, and long

relaxation times are related to free water in the cells (Gusta et al., 1979; Bacic and Ratkovic, 1984; Balla et al., 1985; Colirc et al., 1988). However the percentage of nonfreezing water (14% to 24%) determined by DSC in our experiment was larger than that of WS (3% to 12%) except in late March. Moreover, the percentage of nonfreezing water decreased from December to March, which did not correlate with the change in WS percentage. WS may not be nonfreezing water, but a form of freezing water like WL. We consider T1 of nonfreezing water in the buds to be too short to be detected by NMR measurements in this experiment. The T1S value reached 180 ms, longer than the T1 values of bound water reported in other papers (Gusta et al., 1979; Colirc et al., 1988). This value supports the presumption that WS, as well as WL, is mobile water, though the molecular mobility of WS is more restricted.

Ishida et al. (1987) assigned WS in soybeans to free water in the cytoplasm and WL to free water in vacuoles. According to this hypothesis, our observation on the water in peach flower buds can be explained as follows: the cytoplasm absorbed water more rapidly than the vacuoles, and consequently the percentage of WS increased. Otsuka (1972) reported, however, that vacuoles in the cortex cells of mulberry began to grow in winter and swelled noticeably during ecodormancy. The vacuoles in the peach flower buds were also expected to swell in ecodormancy, though WL decreased in that period. Thus, we could not show that WL was water in vacuoles. We suggest that WL and WS are distributed in the whole tissue.

The change in the T1L value corresponded to the progress in the developmental stage during ecodormancy as well as the change in the water percentage as to the fresh weight. This suggests that the increase in T1L was derived from a decrease in the viscosity of the cellular water caused by water absorption by the cells. The water proton T1 has generally been reported to be closely correlated with the water content in plant tissues (Gusta et al., 1979; Kaku et al., 1984; Colirc et al., 1988). Thus, T1L of flower buds can be used as an indicator of the physiological development in ecodormancy dominated by water absorption.

Although a remarkable change in T1L was not observed during endodormancy, T1S increased at a constant rate from shortly before the rest break until late February. This suggests that T1S may have sonic relation with the physiological change at the rest break.

The water proton relaxation time measured by NMR reflects the viscosity of the cellular water, which depends upon water absorption by the cells. Its change also corresponds to progress in the developmental stage in ecodormancy. DSC data afford us information on changes in nonfreezing water content in flower buds which can not be detected by NMR. Thus, NMR and DSC analyses are useful for estimation of the physiological changes in buds according to the developmental stage.

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